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THE EFFECTIVENESS OF COATED UREA PRILLS AND COPPER TREATED UREA PRILLS AS NITROGEN SOURCES FOR RUMEN MICROORGANISMS[1]

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INTRODUCTION

Urea has been successfully used as a nitrogen supplement in formula rations for ruminants for several years. Often, the proportion used has depended on the existing cost relationship between urea and natural protein supplements such as oil seed meals. However, it has been an accepted practice on the part of animal nutritionists to recommend that urea provide not more than 1/3 of the supplementary nitrogen nor more than one percent in the total ration. Although this recommendation placed a limit on the use of urea, it was based on good experimental findings.

It is quite certain that the majority of the rumen bacteria ultimately utilize ammonia (NH_3) nitrogen in their synthesis of protein in growth processes. If proteins provide the nitrogen source in the ration, they are hydrolyzed and most of the amino acids deaminated before the nitrogen is used in the form of NH_3 by the bacteria. Urea contributes the same product NH_3, through the action of bacterial urease enzymes present in the rumen. However, since urea is very soluble it is immediately subject to attack by urease upon entering the rumen. As a result, the NH_3 level in the rumen rises sharply shortly after consumption of a ration containing urea. Ammonia can also be absorbed through the rumen wall in which case much of it goes to the liver, is converted back to urea and is partly excreted through the kidney. Thus, unless the rumen NH_3 is utilized rapidly by the rumen bacteria, it may be wasted.

It would seem obvious that two phases of research are essential to increasing the utility of urea as a nitrogen supplement.

1) Research on means to hasten the utilization of urea (or NH_3) nitrogen by rumen bacteria.

2) Research on means to slow the release of NH_3 from urea in the rumen.

Most of the research to date has been devoted to the former objective. This has consisted mainly of investigating efficiency of utilization at various dietary levels of urea and using different rations. It was quite obvious from these studies that the faster and more active the fermentation was taking place in the rumen, the faster the incorporation of NH_3. Thus, urea is used primarily in rations containing relatively high amounts of readily fermentable carbohydrates such as starches and sugars.

The second objective has not been given as much attention. This paper presents the results of several investigations designed to determine (a) the release of NH_3 from and (b) rumen bacterial utilization of nitrogen in the form of:

1) Urea prills[4] coated with various fats and waxes.
2) Urea prills compounded with various amounts of $CuSO_4$.

I. STUDIES ON COATED UREA PRILLS

Since urea is highly soluble, one of the obvious ways to attempt to slow its release was to provide urea prills with a coating which would prevent contact with water for some time. Since fats and waxes are insoluble in water and many were commercially available in quantity, they appeared to be logical materials to use in coating urea. Thus, several commercially prepared, wax-coated urea prills[5] were tested for nitrogen availability as described in the following section.

[1]Supported in part by a Grant-in-Aid of research from the Grace Chemical Company, Memphis, Tennessee.

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[4]"Prills" refers to large urea granules formed when urea is dispersed into a "prilling tower".

[5]Prepared by the Grace Chemical Company, Memphis, Tennessee.

PROCEDURE:

NH_3 Release Tests Using Urease Enzyme

The NH_3 release from coated urea prills was tested by the following technique. Sufficient test material to contain 100 mg. of N was weighed into a 150 ml. bottle. To this was added 100 ml. of pH 7.0 phosphate buffer (4.36 g. KH_2PO_4 + 10.59 g. Na_2HPO_4 per liter) containing 100 mg. of commercial Jackbean urease dissolved in the buffer. The buffer had been preheated to 39°C. The contents were immediately sampled by removing a 10 ml. aliquot. The bottle was then placed in a 39°C water bath and further 10 ml. samples were taken at 5, 10, 20, 30 minutes and 1 or 2 hours after the zero time sample. All samples were placed in Kjeldahl flasks previously prepared for NH_3 determination (AOAC method, 1940) by additions of magnesium oxide and calcium chloride. Free NH_3 was determined on the flasks by distillation into saturated boric acid solution and titration with standard HCl.

In Vitro Rumen Fermentations

The in vitro rumen fermentations were conducted according to the procedure described by Bentley et al. (1955) with the exception that urea was removed from the basal medium. This modified basal media supported no microbial growth without additions of nitrogen. Standard dose-response curves were obtained using levels of 0, 10, 20, 30, 40 and 50 mg. of urea-N. Although both cellulose digestion and trichloroacetic acid insoluble nitrogen were determined as measures of microbial activity, the latter figures were used in calculating the efficiency of the test sample to replace urea. TCA-insoluble nitrogen is taken as an indication of bacterial protein (or growth).

RESULTS:

In Vitro Trials

Preliminary work was conducted with urea samples of varying pellet size and density. These samples were tested for rate of solubility and release of NH_3 by urease enzyme. It was apparent from the results that solution of the urea pellets and subsequent hydrolysis by urease was similar to that of crystalline urea. Thus, it appeared that an actual physical masking would be necessary before the solution of the urea could be slowed.

Samples of urea prills coated with 23 different materials were tested for rates of solubility and hydrolysis of the urea contained therein. The various coatings are described in Table 1. The coatings were applied to the urea prills either as hot melts, as syrups or as sprays as indicated in Table 1 and dried by tumbling. When the coating material had a melting point at or near room temperature, the coated prills were dusted with barium sulfate or kaolin.

Table 2 shows the rates of solution and NH_3 release for samples 1 through 10 as compared to crystalline urea. None of the coatings appreciably lowered the rate of solution. Samples 4, 6 and 10 showed the greatest promise as they gave the lowest percent release at 5 and 10 minutes but at 20 minutes, their nitrogen was all released.

Samples 1 through 5 were also compared to crystalline urea as nitrogen sources for the in vitro rumen fermentation. All samples tested compared very favorably with urea on the basis of both cellulose digestion and protein synthesis by the rumen bacteria. Thus, none of these 5 coatings were toxic.

The release of NH_3 from samples 11 through 19 were tested in the same manner. These data are presented in Table 3 (Experiment I and II).

Samples 12, 16 and 17 exhibited considerably slower release of NH_3 than any other samples tested so far. These samples had released only 78, 74 and 54%, resp., of their nitrogen at 30 minutes although all the nitrogen was released by 2 hours. These three samples were tested again along with sample 10 and urea. These data are presented in the NH_3 release data shown in Experiment III, Table 3. Again, the release from sample 10 was only slightly slower than urea. Later solubility tests showed that the slowed release from these samples was due solely to retarding of the solution of the urea and not to inhibition of the urease enzyme.

The utilization of the nitrogen in samples 12, 16 and 17 by rumen bacteria was tested in vitro. The test samples were used both as the sole source of nitrogen and in combination with urea at levels of 20 and 40 mg. N/100 ml. The coated urea samples appeared to be somewhat less efficient than urea as a nitrogen source in one experiment but in a second experiment the coated materials were utilized as well as urea.

Samples 20, 21 and 22 were prepared by combinations of the coating procedures used for samples 16 and 17. The NH_3 release rates from these samples are illustrated in Figure 1. The release of NH_3 from samples 21 and 22 was much slower than from any other samples tested. Only about 60 percent of the NH_3 had been released at 6 hours. The release of NH_3 from sample 20 was faster than from samples 21

TABLE 1. DESCRIPTION OF THE COATINGS USED ON COATED UREA PRILLS

Sample No.	Coating Material	Solvent	Method of Applying Coating
1	Cellulose Acetate	Acetone	Spray (2 coats)
2	Monoglyceride Mixture	Skellysolve F	Spray (4 coats)
3	Cellulose Acetate Butyrate	Acetone	Spray
4	Acetostearin No. 500	Skellysolve F	Spray (4 coats)
5	Hydrogenated Soya Glycerides	Skellysolve F	Spray
6	Polystyrene	Benzene	Spray (2 coats)
7	Shellac	-----	Pouring on and Tumbling
8	Hystyrene S – 97	Skellysolve F	Spray
9	Acetostearin No. 94	-----	Poured, Tumbled, Dusted
10	Microcrystalline Wax	-----	Melt
11	Polyethyene No. 629	-----	Melt
12	Sugar Cane Wax No. 700	-----	Melt
13	Polymekon Wax	-----	Melt
14A	Concord Waxol	-----	Melt
14B	Concord Waxol	Skellysolve F	Spray
15	Myvacet, acetylated Monoglycerides Type 500	Skellysolve F	Spray
16	Castor Wax	-----	Melt
17	Hydro Tallow Glycerides No. 58	-----	Melt
18	Lecithin DL – 1	-----	Poured, Tumbled, Dusted
19	Petrolatum – Rosin – Paraffin 1 – 3 – 1	-----	Melt, Dusted
20	Hydro Tallow Glycerides No. 58	-----	Melt
21	Castor Wax followed by	-----	Melt
	Hydro Tallow Glycerides No. 58	-----	Melt
22	Clay followed by Hydro Tallow Glycerides No. 58	-----	Melt

TABLE 2. THE RATE OF NH_3 – RELEASE FROM COATED UREA PRILLS (1–10) AS DETERMINED BY THE JACKBEAN UREASE TEST

	Incubation time in minutes[1]				
Sample	0	5	10	20	30
Urea	6.6	45.1	77.2	97.6	99.8
1	2.0	34.5	73.6	101.1	104.1
2	2.0	36.4	75.7	103.6	105.6
3 – A (Green)	5.0	48.5	80.0	100.0	101.0
3 – B (Blue)	6.0	46.5	80.0	98.0	99.5
3 – C (Gray)	2.0	41.5	79.5	104.5	104.5
4	1.0	32.0	69.0	104.0	109.0
5	4.0	49.0	85.0	99.5	99.5
6	2.8	28.9	70.0	105.0	105.5
7	6.5	42.0	74.5	96.0	96.5
8	4.0	34.5	72.0	100.5	102.0
9	6.5	38.0	66.0	90.5	94.0
10	2.5	30.0	62.5	95.5	102.5

[1]Ammonia nitrogen released in a given time is expressed as percent of the total urea nitrogen added at zero time.

TABLE 3. THE RATE OF NH_3 - RELEASE FROM COATED UREA PRILLS (11-19) AS DETERMINED BY THE JACKBEAN UREASE TESTS

Sample	Incubation time in minutes[1]					
	0	5	10	20	30	120
Experiment I						
Urea	12.5	68.5	89.0	100.0	-----	-----
11	3.0	21.5	54.0	97.5	100.0	-----
12	0.0	6.7	29.0	58.0	78.5	100.0
13	1.0	19.0	54.0	100.0	-----	-----
14A	4.0	46.5	79.0	100.0	-----	-----
14B	6.0	52.5	82.0	100.0	-----	-----
Experiment II						
Urea	8.0	55.2	85.5	98.0	100.0	-----
15	0.0	11.5	46.8	85.3	100.0	-----
16	0.0	8.5	29.0	57.5	74.5	100.0
17	0.0	3.5	13.0	29.5	54.3	100.0
18	3.5	33.2	73.5	94.5	100.0	-----
19	0.0	13.5	61.5	94.0	100.0	-----
Experiment III						
Urea	8.5	52.5	83.0	100.0	-----	-----
10	1.5	26.5	72.0	100.0	-----	-----
12	0.0	7.5	30.0	59.0	81.5	100.0
16	0.0	10.5	36.5	64.0	84.5	100.0
17	0.0	5.0	16.0	38.5	67.0	100.0

[1]Ammonia nitrogen released in a given time is expressed as percent of the total urea nitrogen added at zero time.

and 22 but was slower than from the first sample made with this coating, i.e., sample 17 (Table 3).

All the tests previously described had been performed using a level of 100 mg. of urease enzyme per 100 ml. of buffer solution which is a relatively high level of enzyme. It was of interest to test the release of NH_3 from one of the coated urea prills using lower levels of urease activity. Consequently, sample 17 was tested with levels of 100, 10 and 1 mg. urease per 100 ml. These data are shown in Figure 2. It can be seen that even though a marked slowing of NH_3 release occurred at the high enzyme level, this effect was considerably less at the 10 mg. level and there was hardly any release at all at the 1 mg. level.

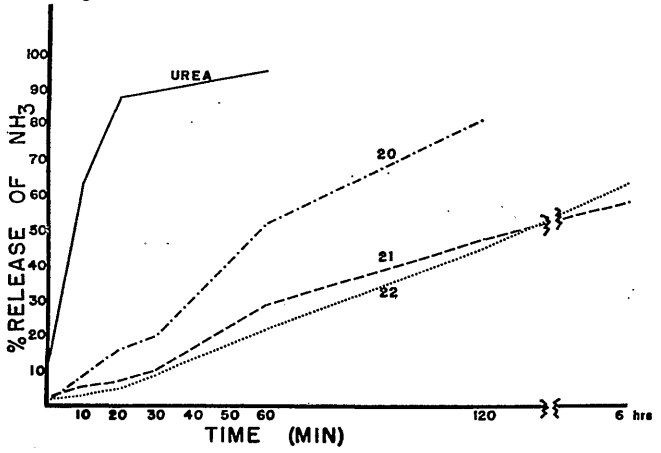

Fig. 1.–Rate of release of NH_3 from samples 20, 21 and 22 using Jackbean urease (100 mg./100 ml.)

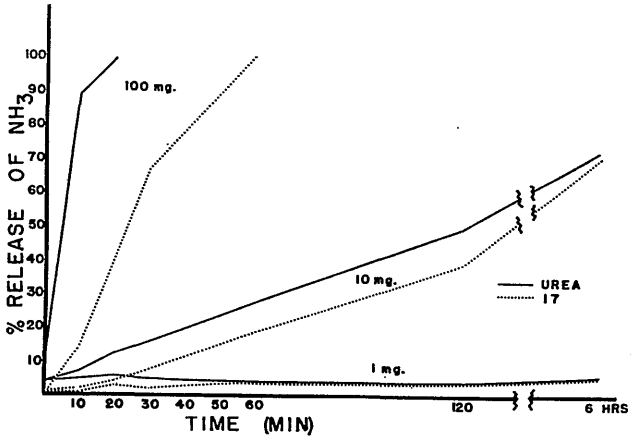

Fig. 2.–Rate of release on NH_3 from urea and sample 17 using 1, 10 and 100 mg. Jackbean urease per 100 ml.

Animal Acceptance of Coated Urea Prills

If the coating of urea prills was to have any practical significance, it was obvious that tests were needed to determine the acceptability of this product to the animal. For this purpose, several short term experiments were conducted using 15 yearling ewes.

EXPERIMENT I

In Experiment I, the animals were divided into 5 lots of 3 ewes each and were fed a ration of hay and one pound of a corn-urea concentrate. The time allowed for eating the concentrate was restricted to about one hour per feed in order to get a fair estimation of the palatability factor. Mixtures of 2, 4 and 6 percent of normal feed urea in the corn mixture were compared to 4 and 6 percent mixtures using coated urea prills from sample 20. The data from this test appears in Table 4. It is readily apparent that restriction of feed consumption time (limited feeding period) markedly reduced the intake of the 4 or 6 percent urea or coated urea prill supplements compared with the 2% urea supplements. Hence, in the second 7 day period, the animals were fed the one pound of concentrate ad libitum. It was found they consumed it all before the next feed for all levels of urea. No difference appeared between the coated and uncoated urea samples.

EXPERIMENT II

In Experiment II, the animals were fed rations similar to those used in Experiment I. However, in this test each lot was simultaneously offered concentrates made both with uncoated or coated urea in separate feeders so that a choice could be made between the two rations. Table 5 shows the 7 day consumption figure comparisons for both 4 and 6 percent urea levels. There did not appear to be any consistent differences between the two forms of urea.

TABLE 4. CONSUMPTION OF UREA CONTAINING SUPPLEMENTS BY LAMBS ON EITHER A LIMITED FEEDING PERIOD OR A FREE CHOICE BASIS[1]

	Weight gain, Average per head, (14 days)	Consumption of Supplement	
		Limited feeding[2] period (7 days)	Ad. Lib. period (7 days)
	lb.	lb.	lb.
Lot 1, 2% urea	2.5	21.0	24.9
Lot 2, 4% urea	3.2	10.5	24.9
Lot 3, 6% urea	1.8	10.4	24.9
Lot 4, 4% coated urea[3]	5.0	11.8	24.9
Lot 5, 6% coated urea[3]	0.3	11.9	24.9

[1]Urea and ground corn supplement.

[2]Supplement for each lot placed in a pan and the lambs given one hour to eat the feed. It was observed that the lambs on the 2% urea-corn mixture ate their feed in 20 to 30 minutes, while the other lots consumed about half of the amount available in one hour.

[3]Sample 20.

TABLE 5. ACCEPTANCE TESTS WITH COATED AND UNCOATED UREA CONTAINING SUPPLEMENTS FOR LAMBS (7 DAYS)

	Feed consumption, lb. per lot of 3[1]	
	Coated-urea[2]	Uncoated-urea
4% corn-urea supplement	18.0	17.0
6% corn-urea supplement	13.1	16.2

[1]The lambs had access to both supplements in separate feeders in each pen. The concentrate was placed in a different feeder each day.

[2]Sample 20.

EXPERIMENT III

Since it was thought that higher levels of corn and urea might better demonstrate differences in palatability, an experiment was designed to test urea levels up to 12 percent of the supplement. The supplement was given to the animals, then additional shelled corn so they were getting a so-called "full feed" of corn. Hay was fed free-choice. The results in Table 6 indicate that the supplement containing 12% urea was not consumed as readily as the other supplements. However, all the animals ate well and appeared healthy in spite of the high urea levels used.

EXPERIMENT IV

A similar experiment was then conducted using coated and uncoated urea at the 8 and 12 percent levels. Again, the animals had access to supplements made with both types of urea. At the end of a 5 day trial, the 8 and 12 percent supplements were reversed for another 5 days. During each 5 day trial the feeders containing the two types of urea supplements were moved at least twice in the pens so the sheep had no opportunity to choose a given feeder as a result of habit.

As can be seen in the data in Table 7, in all cases the supplements containing uncoated urea were highly preferred over those made with coated urea. At this time, no explanation can be offered for this result. Possibly, the larger coated prills were easier for the animals to detect in the supplement and they chose the other on the basis of its physical texture rather than its urea content. It should be borne in

TABLE 6. FEED ACCEPTANCE EXPERIMENT USING DIFFERENT LEVELS OF UREA IN THE FEED SUPPLEMENT FOR LAMBS (TWO-WEEK PERIOD, MAY 24 TO JUNE 7)[1]

Lot number and supplement	Number of lambs	Total gain, lb.	Feed consumption, pounds			
			Supplement	Corn	Hay	Total
Lot 1, 2% urea	3	19.0	19.9	52.6	27.5	100.0
Lot 2, 8% urea	3	20.0	22.0	50.5	33.0	105.0
Lot 3, 12% urea	3	23.0	14.4	78.0	38.0	130.0
Lot 4, 2% urea	6	15.7	27.2	47.3	34.5	109.0

[1]It was noted by the feeder that the lambs in Lot 3 (12% urea supplement) had damp fleeces and the wool had a greasy feel. The bedding was wetter also.

TABLE 7. ACCEPTANCE TESTS USING COATED UREA PRILLS VS. UNCOATED UREA FED AT DIFFERENT LEVELS TO YEARLING EWES (JUNE 8 TO JUNE 18, 1956)

	Feed consumption, pounds[1]	
	Lot 2	Lot 3
	8% urea supplement	12% urea supplement
Supplement with coated urea	1.88	0.76
Supplement with uncoated urea	6.62	2.68
Whole shelled corn	19.08	26.0
Hay	9.0	13.0
	12% urea supplement	8% urea supplement
Supplement with coated urea	1.64	0.22
Supplement with uncoated urea	6.12	7.82
Whole shelled corn	19.50	28.18
Hay	9.00	8.00
Weight gain, average for 10 day period, pound per head	-1.5	3.0

[1]Three ewe lambs per lot. Feed consumption given as total feed consumed by the three lambs in the 5 day period. At the end of the 5-day period, the rations were reversed. The supplement and shelled corn were each in a separate feeder and the lambs were fed ad libitum.

mind that at the lower more practical levels of urea tested earlier, no preferences were demonstrated.

II. COPPER TREATED UREA

A second approach tested the inhibitory effect of copper on the activity of urease enzyme. It was recognized at the start that any material which successfully inhibited urease activity might also inhibit rumen bacteria, as well, or might even be toxic to an animal consuming the material. Nevertheless, the idea seemed worthy of investigation.

Six samples of urea prills formulated with varying amounts of $CuSO_4 . 5H_2O$ were prepared by a commercial urea producer. Their compositions are shown in Table 8.

TABLE 8. COMPOSITION OF SAMPLES 23 - 28

Sample No.	Treatment
23	0.01% $CuSO_4.5H_2O$
24	0.10% "
25	0.20% "
26	0.25% "
27	0.50% "
28	1.0% "

PROCEDURE:

Ammonia release tests and in vitro rumen fermentations were performed in the manner described earlier in this report.

The procedure for the animal feeding trials will be described individually in the results section.

RESULTS:

Hydrolysis of Treated Urea with Jackbean Urease

Figure 3 illustrates the NH_3 release from samples 23 – 28 as compared to pure urea in two different experiments. It is apparent that as the level of copper in the urea prill increases, the rate of urea hydrolysis decreases. The enzyme was very markedly inhibited by samples 27 and 28.

To clarify the nature of the inhibition, sample 25 was tested in similar fashion with three levels of urease enzyme, i.e., 50, 100 and 200 mg. urease per 100 ml. (Figure 4). At the 50 mg. enzyme level, there was a considerably slower release of NH_3 than at the 100 mg. level. At 200 mg. enzyme, the release was faster than the release from pure urea when 100 mg. enzyme was used. This indicates the copper is tying up the enzyme in stoichiometric proportions, confirming previous reports of the effect of divalent metal ions on urease enzyme (Sumner and Somers, 1946).

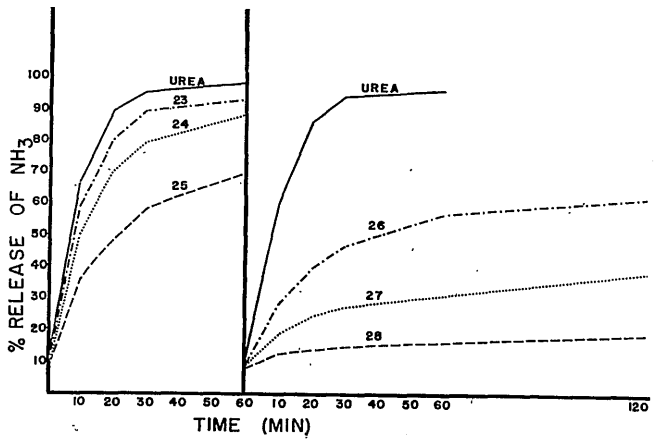

Fig. 3.–Rate of release of NH_3 from samples 23, 24, 25, 26, 27 and 28 using Jackbean urease (100 mg. /100 ml.)

Hydrolysis of Cu-Treated Urea by Rumen Microorganisms

Rumen microorganisms were used as a source of urease enzyme in a medium which allowed for bacterial growth and cellulose digestion. The NH_3 release data from this test are presented in Figure 5. Again, it is apparent that hydrolysis was slower than when urease

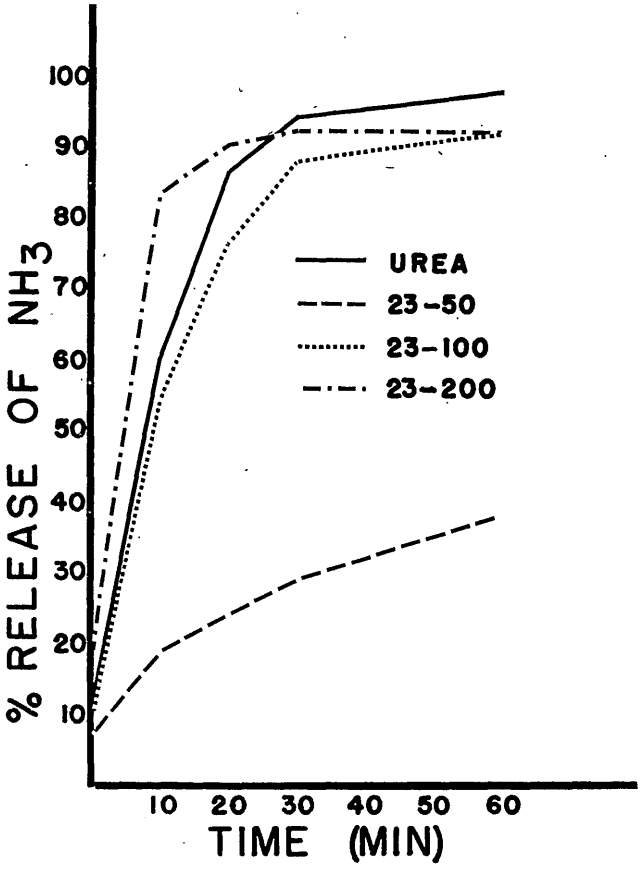

Fig. 4.–Rate of release of NH_3 from sample 23 using 50, 100 and 200 mg. Jackbean urease per 100 ml.

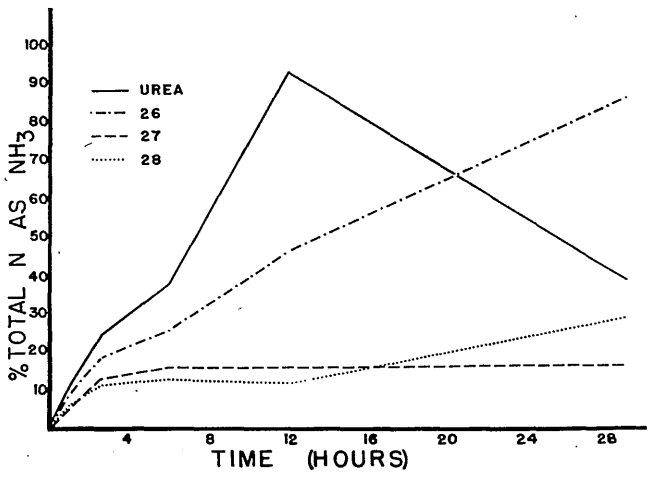

Fig. 5.–Level of free NH_3 in In Vitro rumen fermentation flasks using samples 26, 27, 28 and urea as N-Sources. Each material was added in sufficient quantity to supply 50 mg. N per 100 ml. media.

was used indicating the urease activity of the rumen population as used here was considerably lower than 100 mg./100 ml. It is apparent that copper delayed the release of NH_3 from all samples and with samples 27 and 28, the release was almost completely inhibited. There was no bacterial growth or cellulose digestion with the latter two samples. Although NH_3 was slowly released from sample 26, little growth was observed. The NH_3 level in the flask with urea decreased after 12 hours due to growth and utilization.

An extensive series of experiments were conducted in which the six copper treated urea samples were tested for their efficiency in supplying the nitrogen requirements for the rumen bacteria. Each sample was tested at 3 levels of nitrogen, i.e., 12.5, 25 and 50 mg. N per 100 ml. In each experiment, a standard urea curve was included using levels from zero to 100 mg. of urea-N per flask. Activity was measured by cellulose digestion and synthesis of trichloroacetic acid insoluble protein. The performance at each level of sample was compared to the urea standard curve on both criteria of activity.

Table 9 presents data from three experiments of this type. In these tests, the copper treated urea samples compared quite favorably with regular urea until the level of copper became high enough to be toxic. For example, samples 23, 24 and 25 gave good results at all levels. However, when 26 and 27 were tested at the normally optimum nitrogen level (50 mg.), the amount of copper present became toxic and some inhibition was observed. With sample 28, this inhibition was apparent at both the 25 and 50 mg. levels of nitrogen. If the copper levels are calculated, it can be seen that generally the samples were inhibiting when the level of copper was greater than 0.75 part per million. Almost complete inhibition was shown at 1.0 part per million and yet there was no inhibition at 0.64 part per million.

The cellulose digestion data suggested that lower levels of copper might even be stimulating, but the results were not consistent enough to draw any conclusions. The TCA – insoluble nitrogen data did not show the same trend. Actually, the protein synthesis data shows that protein synthesis tends to be more easily inhibited by the copper than cellulose digestion.

The results in Table 12 illustrate the effect of copper levels when added as pure $CuSO_4.5H_2O$ on the activity of rumen bacteria. Marked inhibition occurred at the 0.4 mg. level of $CuSO_4.5H_2O$ or approximately 1 part per million of copper. These data confirmed the earlier findings on the level of copper which was toxic.

An attempt was made to determine whether the toxicity of the copper was being caused merely by inhibition of the urease enzyme activity or whether the copper was actually being toxic for the bacteria as a whole. In these tests, $(NH_4)_2SO_4$ nitrogen was added to or substituted for the sample nitrogen. It appeared from these limited tests that $(NH_4)_2SO_4$ was only about 60 percent as efficient as urea nitrogen. The presence of NH_4 + nitrogen did not markedly improve the performance at any time. Thus, it appeared that the copper was inhibiting the organisms as a whole and not necessarily just the specific enzyme, urease.

The Use of Cu-Treated Urea in Heifer Rations

Eleven Hereford heifers weighing an average of 691 lb. were used in two short term feeding trials to determine (1) the palatability of high urea supplements and (2) palatability of similar supplements using copper treated urea.

Since the animals had been on a grazing experiment, they were fed increasing amounts of ground ear corn in a preliminary period until they were eating at least 10 lb. per head per day. Then they were divided into three lots of 3 animals each and one lot of 2 animals. In the first trial, the animals were fed 2 lb. per head per day of supplements of the following composition:

	Lot 1	Lot 2	Lot 3	Lot 4
Soybean oil meal	1.5	1.0	0.5	------
Urea, lb.	------	0.08	0.16	0.24
Ground ear corn, lb.	0.5	0.92	1.34	1.76
% urea in supplement	------	4.0	8.0	12.0
Calc., protein, lb.	0.7	0.72	0.74	0.74

TABLE 9. EVALUATION OF COPPER TREATED UREA MICROPRILLS AS N-SOURCES FOR RUMEN MICROORGANISMS IN VITRO

Two grams cellulose substrate

Sample	Mg. level of nitrogen	Percent theoretical[1] cellulose digestion				Percent theoretical[1] TCA-N synthesis			
		8/22/56	8/30/56	9/5/56	Av.	8/22/56	8/30/56	9/5/56	Av.
	12.5		90.4	111.2	100.8		91.2	100.8	96.0
23	25.0	92.0	88.0	101.2	93.7	74.4	86.4	104.0	88.3
	51.2	133.8	73.8	97.1	101.6	78.1	52.4	100.0	76.8
	12.5	144.0	84.8	112.0	113.6	115.2	91.2	104.8	103.7
24	25.0	92.0	80.8	84.8	85.9	51.2	66.8	88.0	78.7
	51.2	102.0	52.0	85.4	79.8	43.0	67.0	86.9	65.6
	12.5	177.6	136.0	64.0	125.9	52.0	97.6	92.0	80.5
25	25.0	208.8	101.2	90.0	133.3	52.8	80.0		66.4
	51.2	140.6	111.0	81.6	111.1	30.3			30.3
	12.5	177.6	130.4	72.0	126.7	72.0	101.6	97.6	90.4
26	25.0	188.0	120.0	74.4	127.5	72.0	72.8	88.0	77.6
	51.2	123.0	65.4	40.2	76.2	43.0		78.1	60.6
	12.5	177.6	144.8	96.0	139.5	65.6	80.0	86.4	77.3
27	25.0	148.0	100.0	80.0	109.3	56.0	75.2	91.6	74.3
	51.2	43.3	15.5	8.7	22.5	20.5	15.3	78.1	38.0
	12.5	184.8	153.6	104.0	147.5	65.5	101.6	86.4	84.5
28	25.0	243.2	52.0	44.8	113.3	54.8		14.0	22.9
	51.2		25.8	7.8	16.8	43.9		26.9	23.6

[1]Based on digestion or activity obtained when an equivalent amount of nitrogen was added as urea in the control flasks.

TABLE 10. THE EFFECT OF VARYING COPPER LEVELS ON CELLULOSE DIGESTION AND TCA-N SYNTHESIS IN THE IN VITRO RUMEN FERMENTATION

Mgms. of $CuSO_4.5H_2O$ per 100 ml.	Gms. cellulose digested		Mgms. TCA-N Synthesized	
	10/2/56[1]	8/28/56	10/2/56[1]	8/28/56
0	0.831	0.844	18.4	17.0
0.05	0.864		18.6	
0.10	0.764	1.03	16.2	19.0
0.15	0.798		19.4	
0.20	1.058	0.855	22.4	20.0
0.25	1.072		23.9	
0.4		0.012		3.0
0.6		0		3.0
1.0		0		1.5
2.0		0		2.5
5.0		0		2.5

[1]Duplicate flasks were run in this experiment and the results averaged.

In addition, they were fed a full feed of ground ear corn and hay. Water, salt and minerals were provided free choice.

After 56 days on the first trial, the supplements for the second trial were changed as follows:

Lot 1	12% sample 28	(1% $CuSO_4.5H_2O$)
Lot 2	12% sample 27	(0.5% $CuSO_4.5H_2O$)
Lot 3	12% sample 26	(0.25% $CuSO_4.5H_2O$)
Lot 4	12% urea	(No $CuSO_4.5H_2O$)

The supplements were formulated similar to that for lot 4 of the first trial. Again, a full feed of ground ear corn and hay were provided in addition to the supplements.

In both phases, the time required to finish eating the protein supplement was noted and recorded. The data from both trials are shown in Table 11.

It was obvious in the first trial that the heifers readily accepted the protein supplement with up to 8 percent urea in it. However, when the urea was raised to 12 percent, it took on the average of 104 minutes to eat the protein supplement as compared to

TABLE 11. PERFORMANCE OF HEIFERS FED SUPPLEMENTS OF SOYBEAN OIL MEAL, UREA OR COPPER TREATED UREA WITH A FATTENING RATION

Supplement by Lots

	Lot 1	Lot 2	Lot 3	Lot 4
TRIAL 1	75% Soy 25% Corn	4% Urea 50% Soy 40% Corn	8% Urea 25% Soy 67% Corn	12% Urea 0 Soy 88% Corn
No. of heifers	2	3	3	3
Av. starting wt., lb. (7/27/56)	637	721	721	685
Av. daily gain, lb. (56 days)	3.10	2.83	2.93	2.61
Av. daily ration, lb.				
Corn	10.6	11.9	11.9	11.9
Protein Supp.	2.0	2.0	2.0	2.0
Hay	9.6	9.6	9.6	9.6
Av. time required to eat protein Supplement, min.	7.0	10.0	12.0	104.0
Total feed required per lb. gain, av. lb.	7.34	8.30	8.0	9.0
TRIAL 2				
Above lots changed to rations shown here (Sept. 24, 1956)	12% Urea with 1% $CuSO_4.5H_2O$ 88% corn	12% Urea with 0.5% $CuSO_4.5H_2O$ 88% corn	12% Urea with 0.25% $CuSO_4.5H_2O$	12% Urea (No Cu) 88% corn
Av. starting wt., lb.	730	770	773	744
Av. daily gain, lb. (42 days)	2.10	1.88	1.71	1.69
Av. daily rations, lb.	12.9	14.2	14.1	14.3
Corn	1.5	2.0	2.0	1.9
Protein Supp.	7.8	7.3	7.3	7.3
Hay				
Av. time required to eat protein Supplement, hrs.	6-9	6-9	6-9	6-9
Total feed required per lb. gain, av.	10.97	12.51	13.67	13.89

12 minutes for the 8 percent urea supplement. Treating the urea with $CuSO_4.5H_20$ as was done in Trial 2 did not particularly enhance its palatability. There were no obvious toxic effects of the levels of copper used.

Since there were so few animals in each lot and the feeding periods were short, no conclusive statements can be based on the rate of gain data. However, it should be noted that the heifers gained well throughout the two trials. In the first trial, the animals on 12 percent urea gained slightly slower than the other animals. The significance of the gain figures in Trial 2 is questionable.

The Use of Cu-Treated Urea in Lamb Feeding Trials

Copper treated urea was tested in two lamb feeding trials. In the first trial involving small numbers of animals the basal ration was deficient in protein (7.16 percent crude protein). Five lots of 6 or 7 lambs each were fed the rations shown in Table 12. All the lambs received an implant in the jaw containing 4 mg. estradiol and 160 mg. progesterone ("Synovex") at the beginning of the experiment to serve as a growth stimulant. In this experiment copper treated urea was compared to untreated urea for lambs on a protein deficient basal ration. In addition, molasses was used as a supplement because of its known content of minerals and other factors which have been shown to exert some beneficial effects on rumen microflora.

The data are shown in Table 13. A marked response in growth and feed saving was obtained by supplementing the basal ration with any of the urea supplements. The lots receiving copper treated urea (4 and 5) gained only slightly faster and slightly

TABLE 12. RATIONS FOR LAMB FEEDING TRIAL 1.

Ingredients	Lot 1 Basal	Lot 2 2% Urea	Lot 3 2% Urea + Molasses	Lot 4 2% Cu-Urea (0.25% Cu)	Lot 5 2% Cu-Urea (0.5% Cu)
Alfalfa meal	20.0	20.0	20.0	20.0	20.0
Ground Corn Cobs	20.0	20.0	20.0	20.0	20.0
Ground Shelled Corn	46.4	44.4	41.9	44.4	44.4
Corn Starch	5.0	5.0	5.0	5.0	5.0
Corn Sugar	5.0	5.0	2.5	5.0	5.0
Animal Fat	2.0	2.0	2.0	2.0	2.0
Calcium Hydrogen Phosphate	0.6	0.6	0.6	0.6	0.6
Trace Mineralized Salt	1.0	1.0	1.0	1.0	1.0
Urea Prills	-------	2.0	2.0	---------	---------
Blackstrap Molasses	-------	-------	5.0	---------	---------
Cu-Urea (0.25% $CuSO_4.5H_20$)	-------	-------	-------	2.0	-------
Cu-Urea (0.5% $CuSO_4.5H_20$)	-------	-------	-------	---------	2.0

TABLE 13. PERFORMANCE OF LAMBS ON UREA AND CU-UREA RATIONS TRIAL 1

LOT	1 Basal	2 Urea	3 Urea Molasses	4 Cu-Urea 0.25%	5 Cu-Urea 0.5%
No. in lot	6	7	6	6	6
Av. wt. Nov. 9	69.7	68.4	69.3	66.5	70.5
Av. wt. Feb. 13	93.6	103.4	112.8	105.4	107.8
A. D. G. 96 days, lb.	.25	.37	.45	.41	.39
Av. daily ration, total, lb.	2.21	2.80	3.25	3.03	2.89
Feed req. per lb. gain, lb.	8.88	7.62	7.17	7.48	7.41
Carcass Grade (Swift & Company) 30 = Prime 40 = Choice 50 = Good		45.3	44.7	45.7	47.7

more efficiently than the straight urea fed lot. The lot receiving molasses performed considerably better than the remaining lots. No explanation can be offered for this other than a possible increase in palatability. Molasses has often been shown to be of special benefit in urea rations. Again, the levels of copper fed did not appear to be toxic.

A second lamb feeding trial was conducted in which large groups of lambs were fed pelleted fattening rations. In this test copper treated urea was compared to urea and soybean oil meal as a source of protein. Sixty-four lambs previously used in a pasture trial were divided by weight into 3 lots which were fed the rations shown in Table 14. The complete rations were pelleted and no additional feed was offered. The rate of gain and feed data are given in Table 15.

There was little difference in the performance of the three groups of lambs. Although the lambs on copper treated urea gained slightly less this difference was not significant. It should be noted that the urea supplemented animals did perform about as well as the soybean oil meal supplemented group.

DISCUSSION:

As was stated earlier the extreme solubility of urea presented certain practical problems in animal feeding. This was even more apparent in observing the characteristics of coated urea prills. It was noted that if there was even a small hole or crack in the coating which would allow water to enter, the urea would dissolve and diffuse out of the coated prill leaving a "shell" of the coating material. It was apparent then that the coating material would have to have physical properties which would allow it to be applied uniformly around the prill and yet dry to a solid, not too brittle coating.

On the first twenty samples tested, only three appeared to have properties which satisfied these criteria. Samples 12, 16 and 17 released NH_3 considerably slower than urea or any of the other samples tested. Later tests showed that this retardation of release was due solely to limiting the access of water to the urea. The coating materials themselves were not inhibiting the urease enzyme activity. The same phenomenon seemed to be true when the coated prills

TABLE 14. RATIONS FOR LAMB FEEDING TRIAL 2.

Ingredients	Lot 1 Urea	Lot 2 Cu-Urea (0.25% $CuSO_4.5H_2O$)	Lot 3 Soybean Meal
Soybran Flakes	200	200	200
Ground Ear Corn	767.7	767.7	704.1
Soybean Meal	----	----	78.6
Urea	15.0	----	-----
Cu-Urea (0.25% $CuSO_4.5H_2O$)	-----	15.0	------
Yeast Premix	0.5	0.5	0.5
Aurofac 10	1.0	1.0	1.0
Vit. A Oil (10,000 I. U. /gm.)	0.5	0.5	0.5
Deflourinated Rock Phosphate	10.0	10.0	10.0
Salt	5.0	5.0	5.0
Trace Mineral Mixtures	0.3	0.3	0.3
	1,000.0	1,000.0	1,000.0

TABLE 15. PERFORMANCE OF LAMBS IN TRIAL 2

	Lot 1 Urea	Lot 2 Cu-Urea (0.25% $CuSO_4.5H_2O$)	Lot 3 Soybean Meal
No. Lambs	21	22	21
Av. Daily Gain (49 days)	0.387	0.353	0.387
Av. Daily Ration, lb.	3.84	3.64	3.76
Feed req./lb. gain, lb.	9.9	10.3	9.7

were tested with rumen bacteria, i.e., rate of solution was slowed but the coatings did not appear to be toxic.

It is important to point out that if the urease activity is too low, the rate of solution even from the coated prills may exceed the urea hydrolysis. Thus the coating would offer no advantage over crystalline urea itself. Whether this is the case with rumen liquor has not been exactly determined. Mills et al. (1942), Wegner et al. (1940, 1941, 1941a) and Stallcup and Looper (1958) reported that NH_3 production in the rumen generally reached a peak at 1 hour after feeding rations containing urea. This would indicate that urease activity was indeed quite high and that some benefit might be obtained by using coated prills.

When the coated prills were compared to uncoated prills in animal acceptance tests, they compared favorably at low levels in the feed supplement. However, when tested in a 12 percent urea supplement, a high preference toward the uncoated urea was shown. Although few supplements contain this high a percentage of urea, it is conceivable that future circumstances may make it practical to make such supplements. If so, the palatability question will be a very important one. As has been said, no explanation can be offered for the refusal of the coated prill supplement other than possibly the large size of the prills.

The $CuSO_4$ in the copper treated prills presented a true inhibitor of the urease enzyme. Certainly, the data showed that it was indeed inhibitory both to pure urease enzyme and to rumen bacteria themselves. It would appear from the in vitro tests that those levels of $CuSO_4$ which slowed urea hydrolysis markedly were also very near the toxic level for the rumen bacteria. The danger of this sort of relationship is quite apparent if one realizes that any advantages of slower urea hydrolysis would be quickly nullified if the bacterial activity in the rumen were retarded.

Nevertheless, the heifer trials showed reasonable acceptance and no obvious toxicity for several levels of the $CuSO_4$ treated urea. Similarly, there were no adverse responses to the $CuSO_4$ treated urea in the lamb trials.

Even though it was shown that NH_3 release from urea prills could be slowed by both coating with waxy coating and treating with $CuSO_4.5H_2O$, there was still no indication that these materials were more efficiently utilized by the ruminant animal. Further in vivo studies will be necessary to prove that point.

SUMMARY:

Twenty samples of urea prills coated with fat and waxy type materials and six samples of urea prills treated with $CuSO_4.5H_2O$ were tested for rate of ammonia release by urease enzyme, utilization by rumen bacteria in vitro and acceptability by both cattle and sheep.

Three coating materials were found to reduce the rate of ammonia from urea prills. These were Sugar Cane Wax No. 700, Castor Wax and Hydro Tallow-Glycerides No. 58 or combinations of these. The coated prills were utilized by rumen bacteria in vitro but were not as acceptable as urea in 12 percent urea supplements for lamb rations.

The $CuSO_4.5H_2O$ in the copper treated prills inhibited urease activity and rumen microorganisms to a degree dependent on the quantity of $CuSO_4$ in the prills. Animal acceptance and growth tests using both cattle and sheep showed no differences between copper treated and regular urea prills.

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